

112288

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 June 2006 (22.06.2006)

PCT

(10) International Publication Number
WO 2006/063625 A1

(51) International Patent Classification:
B03C 5/02 (2006.01)

(21) International Application Number:
PCT/EP2004/053591

(22) International Filing Date:
17 December 2004 (17.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant (for all designated States except US): **AGILENT TECHNOLOGIES, INC.** [US/US]; 395 Page Mill Road, Palo Alto, California 94306 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **PRECHEL, Tobias** [DE/DE]; Dusenbacherstr. 15, 76337 Waldbronn (DE). **ELGASS, Helmut** [DE/DE]; Falkensteinstr. 25, 76307 Karlsbad (DE).

(74) Agent: **BARTH, Daniel**; Herrenberger Str. 130, 71034 Böblingen (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

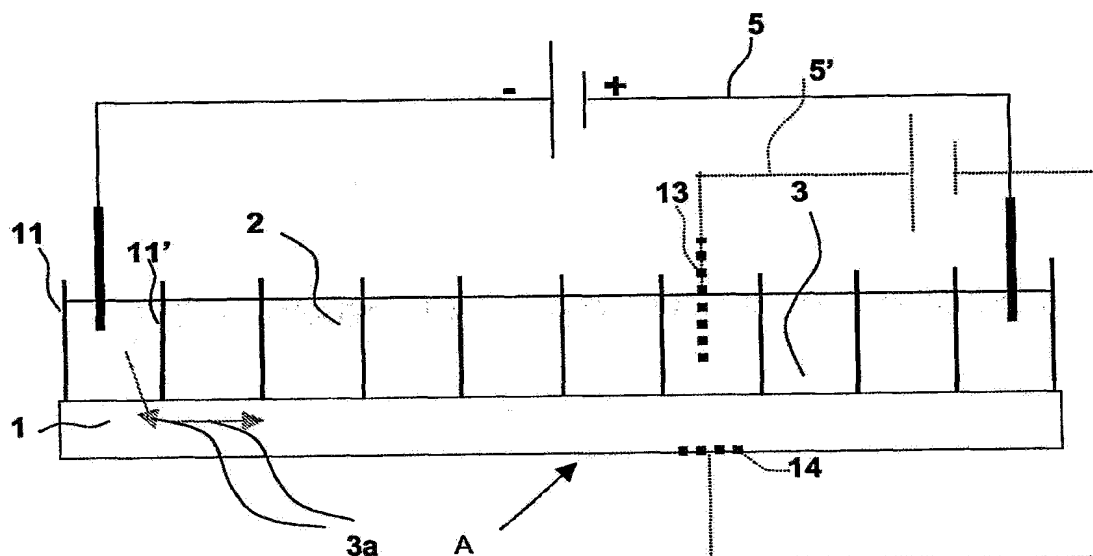
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FRACTIONATION USING ELECTRO ELUTION



(57) Abstract: A device for separation (A) of charged molecules (7), comprising an elution device comprising a fluid, a separation medium (1) being in contact with the fluid, a first electrode (5) adapted to subject the charged molecules (7) to motion within the separation medium (1), and at least one second electrode (5') adapted to subject the charged molecules (7) to motion from the separation medium (1) into the fluid.

WO 2006/063625 A1

FRACTIONATION USING ELECTRO ELUTION

BACKGROUND ART

[0001] The present invention relates to an improved elution of molecules.

5 [0002] A large variety of separation and purification systems is known in the art to separate and identify biological samples which often contain up to 30,000 different proteins. Some of the most popular separation techniques are isoelectric focussing (IEF), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), or native gel electrophoresis (e. g. Blue Native, BN-PAGE).

10 [0003] Furthermore, combining BN-PAGE and SDS-PAGE is known in the art: Separation of proteins by gel electrophoresis can be performed under native or denaturing conditions. BN-PAGE is a charge shift method, in which the electrophoretic mobility of a protein/protein-complex is determined by the negative charge of the bound Coomassie dye and the size and shape of the
15 protein or protein complex. Coomassie does not act as a detergent and is reported to preserve the native structure of oligomeric protein complexes. The resolution of BN-PAGE is much higher than that of gel filtration, sucrose density gradient centrifugations or other gel electrophoresis methods.

[0004] Complexes resolved by BN-PAGE can further be separated by a
20 second dimension SDS-PAGE, their constituents can be visualized. (Schamel, WWA., *Biotinylation of Protein Complexes may lead to Aggregation as well as to Loss of Subunits as Revealed by Blue Native PAGE*. J Immunol Meth 2001, 252, 171-174.

[0005] A combination of IEF and SDS-PAGE results in the two-dimensional
25 gel electrophoresis: In a first dimension, separation is performed subjecting the amphoteric proteins to isoelectric focussing using immobilized pH gradients (IPGs), accordingly leading to a separation by isoelectric point within a pH gradient. Iso-electric focussing systems are usually free flowing buffered

systems or immobilized buffered systems using IGP's for "trapping" the compounds of interest in the gel. The compound of interest is recoverable in fluid phase then under increased efforts, see WO 03/50651 for example.

[0006] In a second dimension SDS-PAGE leads to separation according to the size of the molecules. SDS is an anionic detergent which binds to proteins at a constant ratio of amino acids / SDS molecules. The charge of the bound SDS molecules is proportional to the protein's size and exceeds the charge of the protein. Anionic micelles are composed, having a substantially constant net-charge per mass unit. Subsequent to the separation recovery of the molecules identification is carried out.

[0007] Further analysis of the proteins by analytical technique, e.g. mass spectrometry, require the recovery of the separated molecules from the respective separation medium after performance of the separation, which still is a costly procedure, requiring the scientist to carry out a series of steps such as detecting the molecules or molecule bands in the separation media, excising the molecules or molecule bands and, finally, converting them into a liquid phase in order to prepare them for further separation or experimentation.

[0008] Depending on the amount of interesting material needed for further analysis, techniques are requested with minimized separation times, offering a product with a high degree of purity. In particular, protein research is a domain requiring simple and fast separating procedures in order to allow researchers to focus purposefully and economically on the few critical proteins involved in a specific disease, e. g.

[0009] Various methods and apparatus for electrophoretic separation of molecules are meanwhile well known in the art, see for example WO 01/86279 A1 to Faupel, M. et al.

[00010] Recently, a method and apparatus for separating an analyte was described by Michel, P. et al. (WO 03/019172 A2), referring to differentially

separating charged and neutral compounds and recovering them in solution.

[00011] Furthermore, a novel means to extract molecules utilizing a separation frame is referred to by WO 03/50652.

DISCLOSURE OF THE INVENTION

5 [00012] It is an object of the present invention to provide an improved separation and elution of molecules. This object is solved by the independent claims. Preferred embodiments are shown by the dependent claims.

[00013] Thus, embodiments of the present invention provide a fast and easy to use elution of molecules from a separation medium after foregoing
10 separation. The molecules of interest, in particular proteins, are recovered in fluid phase and, accordingly, subsequent identification or any desired proceeding is performed easily.

[00014] In one embodiment the present invention provides a device for separation and electro elution of charged molecules, having a separation
15 medium in order to carry out separation of the molecules firstly and an elution device which stands in direct communication with said separation medium. Herein, the separation is based on 2-D electrophoresis. An electrode couple contacts the separation medium in order to provide separation based on electrophoresis within the layer of said separation medium and one or more
20 additional electrode couples provide a current flow between the separation medium and the elution device, thus causing molecules to move from the separation medium into the elution device when voltage is applied, accordingly performing "electro elution" of the molecules.

[00015] In another embodiment of the present invention again a device for
25 separation and electro elution of charged molecules is provided, in contrary to the above embodiment being based on the off-gel electrophoresis technique, comprising separation by use of an immobilized pH-gradient gel. Electro elution of this embodiment is performed in analogy to the above embodiment.

[00016] Further embodiments refer to the design and material of the separation medium, the elution device and the arrangement of both with respect to each other and with respect to the first and second electrode pairs.

5 [00017] An additional embodiment takes into account that oxidation of proteins due to the contact with electrodes is to be prevented.

[00018] Furthermore, a method is provided teaching the elution of separated molecules by use of the embodiments of the device of the present invention.

10 [00019] Additionally, an embodiment of the present invention refers to a processing system for separation and processing of charged molecules, thus combining the device for separation and elution of charged molecules or, respectively, proteins, with withdrawing and/or processing devices permitting further processing of the eluted fractions of proteins in order to allow separation, elution, and characterisation of the molecules of interest in one continuously performed proceeding.

15 [00020] Accordingly, methods are provided teaching separation, elution and further processing such as advanced separation or characterization of the molecules of interest.

BRIEF DESCRIPTION OF DRAWINGS

20 [00021] Other objects and many of the attendant advantages of embodiments of the present invention will be readily appreciated and become better understood by reference to the following more detailed description of preferred embodiments in connection with the accompanied drawings.

25 [00022] FIG. 1a is a side view of one embodiment of the device of the present invention, depicting the step of separation after SDS-PAGE or BN-PAGE,

[00023] FIG. 1b is a side view of one embodiment of the device of the present invention, depicting the step of separation as according to the off-gel

technique,

[00024] FIG. 2 shows the device of FIG.1a, depicting the step of elution in the initial state,

[00025] FIG. 3 shows the device of FIG.1a, depicting the step of elution
5 in the finished state for one compartment,

[00026] FIG. 4 is a side view of another embodiment of the device of the present invention, showing a plurality of electrodes couples as used for the step of elution.

[00027] FIG. 5 is a schematic side view showing the processing system
10 comprising the device for separation.

[00028] Before the invention is described in detail, it is to be understood that this invention is not limited to the particular component parts of the devices described or to process steps of the methods described, as such devices and methods may vary. It is also to be understood, that the terminology used herein
15 is for purposes describing particular embodiments only and it is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms of "a", "an", and "the" include plural referents until the context clearly dictates otherwise. Thus, for example, the reference to "an electrode couple" may include two or more such electrodes couples, if it is
20 reasonably in the sense of the present invention.

[00029] Features that are substantially or functionally equal or similar will be referred to in the accompanying figures with the same reference numerals.

[00030] Generally, the separation of molecules, in particular of proteins, is carried out by use of one of the separation techniques such as isoelectric focussing (IEF), sodium dodecyl sulphate polyacrylamide gel electrophoresis
25 (SDS-PAGE) or native gel electrophoresis (e. g. Blue Native, BN-PAGE). All those techniques have in common that they are carried out by use of a

separation gel, and they make use of the properties of molecules, in particular of proteins, to carry a charge, to become charged or to be bound in a complex which is charged.

5 [00031] Accordingly, the above techniques or combinations thereof are performed applying a solvent wherein a mixture of molecules to be separated is contained, to a separation gel or polymer or, generally said, to a compartment. Now, the solvent carrying the molecules or proteins, respectively, is forced to flow within a gel provided with an immobilised pH-gradient (IPG-gel) which may be brought up onto a carrier substrate.

10 [00032] A gel provided with an immobilised pH-gradient (IPG-gel) can be combined with an electrode couple to perform a pre-fractionation of proteins, for example: All molecules or groups of molecules having the same isoelectric point (pI) move along the same distance within the IPG gel layer when voltage is applied, which is generally done by use of an electrode couple with the
15 electrodes located distant from each other, at opposite places in contact with the separation gel.

[00033] The separation effect due to the pI is optimised when a liquid layer is placed above the separation layer, the liquid layer being electrified and thus causing a movement of the molecules off the gel into the liquid and back into
20 the gel, until they reach the point of the gel where the pH-value of the gel corresponds to their isoelectric point (see FIG. 1b).

[00034] This so-called "off-gel electrophoresis" provides among other possibilities a proteomics research tool for studying different proteins, enabling scientists to perform micro-scale separation of proteins and peptides in
25 complex mixtures according to their isoelectric points, grouping them by their common electrical properties. A simple and fast 'filtering' procedure is provided.

[00035] Combining the above off-gel system with the elution technique of the

present invention, which may be called "electro elution", allows for automated multi-dimensional separations of complex protein mixtures.

[00036] A second option is combination of 2-D gel-systems with the elution technique of the present invention, leading to the same result of separation and
5 an enhanced elution of molecules (see FIG.1a).

[00037] By use of the device of the present invention, preferably in combination with the above gel systems, the molecules separated or pre-fractionated can be recovered quantitatively in liquid phase, thus being ready to use for further procedures such as identification or experimentation.

10 [00038] Referring now to FIG. 1a, the device of the present invention is shown, which provides additionally to a separation an enhanced elution for charged molecules 7. It has a separation medium 1 which is designed as a separation strip herein. Of course, another design such as a layer is possible.

[00039] The separation medium 1 herein comprises a separation gel which
15 is adapted to perform SDS-PAGE or BN-PAGE.

[00040] FIG. 1b shows another embodiment of the device of the present invention, which is substantially constituted as the one depicted in FIG. 1a, wherein a difference results from the fact that the separation medium 1 herein comprises an IPG separation gel which is adapted to perform IEF.

20 [00041] Generally, in the device of the present invention an elution device is provided which comprises an eluent 2. The elution device is composed of frame 11 which borders the eluent 2, furthermore it is subdivided in compartments 11' for separation of the frame 11 into different compartments. The element which serves to form the compartments 11' is a grate-like element,
25 it is adopted to the surface area of the separation medium 1. The grate-like element may be fixed at the frame or it can be loose, accordingly it can be laid into the frame 11 at any time.

[00042] The separation medium 1 is in direct contact with the eluent 2 since the elution device is arranged immediately above the separation medium 1, thus allowing molecules 7 to move from the separation medium 1 into the eluent 2 in order to be recovered.

- 5 [00043] The eluent 2 is generally an appropriate fluid or, respectively, a solvent for protein elution such as a buffer which corresponds with the gel-material of the separation medium 1. It is subdivided in portions by the grate-like element when it is filled in the frame 11.

- 10 [00044] Advantageously, the eluent is adapted to the gel of the separation medium, accordingly one would choose an IPG-buffer when the embodiment of **FIG. 1b** is chosen, whereas a different appropriate solvent is selected to serve as eluent for proteins which are eluted in the device according the embodiment shown in **FIG. 1a**.

- 15 [00045] All of the accompanying figures show clearly that the device of the present invention has a first electrode couple 5 which is arranged in a way that the charged molecules 7 are subjected to motion along the separation medium 1: The electrode couple 5 is located a opposite ends of the device of the present invention, both of the electrodes herein being immersed in the eluent and, accordingly, providing an electrical current when voltage is applied.

- 20 [00046] The device of the present invention can be charged with the solvent containing the molecules 7 of interest by filling it into a compartment 11' next to the frame, as indicated in **FIG. 1a**. Applying voltage on the electrode couple 5 causes the molecules to move unidirectionally along the separation medium 1, with respect to its longitudinal expansion (see the arrows 3). The separation
25 medium 1 can be an SDS- or BN-gel or another gel to perform native gel- or denaturing electrophoresis. When the technology is based on performing SDS-PAGE or BN it has to be taken into consideration that there is no equilibrium state achieved: Accordingly applying voltage on the electrode couple 5 - "first electrophoresis" - has to be stopped after a definite operating time, then the

second step can be performed. One could add an appropriate dye or fluorescent dye to indicate the state of electrophoresis in order to know precisely the optimal time for interrupting said first step.

[00047] On the other hand in **FIG. 1b** the molecules move in dependence of
5 their pI , which corresponds with the respective pH of the separation medium 1 being an immobilized pH gel or the like. The pH of the gel herein increases as indicated by the arrow 6. Accordingly, molecules can move in bi-directionally within the strip-like separation medium with respect to its linear extension. Herein, the molecules pass alternating gel and eluent due to the isolating
10 grate-like elements until an equilibrium is achieved and therefore the molecules "remain" on their places, ready to be subjected to the second step being the electro elution :

[00048] Most important for the enhanced elution is the second electrode couple 5', which is provided to subject the charged molecules 7 to motion in
15 vertical direction with respect to the plane of the separation medium 1. The molecules are directed due to electronic forces to get "off" the separation medium 1 into the eluent 2, as can be seen in **FIG. 2**, indicating the movement by the arrow 8, and in **FIG.3**.

[00049] These figures point out that operating the second electrode couple
20 5', which has a first electrode 13 being immersed in a compartment 11, and a second electrode 14, which is in contact with a portion of the separation medium 1 being directly located below said compartment 11' leads to an electric circuit when voltage is applied. Due to this electric current the charged molecules get off the gel and elution takes place.

25 [00050] The voltage may be maintained until the molecules are recovered quantitatively off the gel or, alternatively, the voltage can be interrupted at any time to take of a sample for further proceeding.

[00051] As can be seen in **FIG. 4**, the principle of electro elution can be

realized with a number of second electrode couples 5', providing a rapid and quantitative elution. The number of electrode couples 5' can be connected in series, if desired.

5 [00052] The electrode couple 5 which is shown in the accompanying figures are shown by use of dashed lines when the elution process is running and, accordingly, the electrode couple 5 is inactive (see FIG's 4 to 5). Vice versa, the electrode couple 5' is shown by use of dashed lines when not in process.

[00053] Since there exists permanently an electrical field the molecules and complexes remain charged during the process of separation and elution, which
10 is an advantageous improvement and which provides an easy mobilization.

[00054] In order to prevent oxidation or reduction of molecules, the electrodes which are immersed into the liquid phase or eluent, respectively, can be covered with an appropriate protective film.

[00055] Furthermore, one may wish to handle the separation medium 1 and
15 the elution device separately, as for example in case of performing SDS-PAGE or BN. It may be desired to complete said "first electrophoresis" before the second step is initiated. Accordingly embodiments may be designed which allow a succeeding composition of the device of the present invention allowing to mount the frame 11 onto the separation device when the separation process
20 is already completed.

[00056] Summarizing it can be said, that a method is provided allowing elution of the charged molecules 7 off the separation medium 1 into the eluent 2 which is contained by the elution device of the device of the present invention. Generally, a separation step is performed before elution is carried
25 out. This is to be done by applying the mixture of molecules on the separation medium 1. The separation is electronically supported. by operating the first electrode couple 5.

[00057] In order to perform elution subsequent so separation a second

electrodes couple providing a first electrode 13, which becomes immersed into the eluent which is filled into the elution device, and a second electrode 14, which is placed opposite to the first electrode 13 and thus at the underside of the first separation medium, is operated. Applying voltage on said first and
5 second electrodes 13,14 causes a vertical motion of the molecules, which are accordingly eluted off the separation medium 1 into the eluent 2. Due to the maintenance of voltage during the separation and elution the molecules 7 or molecule complexes may remain charged.

[00058] In order to permit a continuous separation, elution and further
10 processing, a processing system B combining said device for separation A and processing of charged molecules with at least one withdrawing device 20 is described in the following invention. It is schematically illustrated in FIG. 5.

[00059] A withdrawing device 20 is provided to withdraw the eluted fractions from the elution device, which is comprised in said device for separation A, as
15 schematically indicated by the arrows 21. Such a withdrawing device 20 could comprise or could be a pipette or a suction hose or any device suitable for transferring a fraction of eluted molecules into a further processing device, or into a first of a series of processing devices, which may be comprised by the processing system (B), too. Preferably, the withdrawing device 20 is arranged
20 in a way that it may be immersed in the eluent.

[00060] A processing device can be a desalting device. Another processing device may be designed to allow protein digestion, in particular trypsin based protein digestion. An additional processing device can be designed to provide concentration of the fraction of eluted molecules. In order to characterize the
25 desalted, digested and concentrated proteins, an analytical device such as a high performance liquid chromatography mass spectroscopy device (HPLC-MS) a 2-dimensional gel electrophoresis system or a protein array can be arranged downstream to said foregoing devices.

[00061] Alternatively to said characterization it may be desirable to perform

another separation in order to increase the state of purity of the separated proteins. Then, the processing system B comprises a processing device being a separation device for further separation such as an electrophoretic separation device, in particular an electrophoretic separation device based on
5 off-gel electrophoresis.

CLAIMS

1. A device for separation (A) of charged molecules (7), comprising:
an elution device comprising a fluid,
a separation medium (1) being in contact with the fluid,
5 a first electrode (5) adapted to subject the charged molecules (7) to motion within the separation medium (1),
at least one second electrode (5') adapted to subject the charged molecules (7) to motion from the separation medium (1) into the fluid.
- 10 2. Device for separation (A) according to claim 1, wherein the fluid is at least one of an eluent (2) and an electrolyte.
3. Device for separation (A) according to claim 1 or any one of the above claims, wherein the separation medium (1) is a layer or a strip, comprising at least one of the following:
15 - a gel or polymer providing an immobilized pH-gradient to perform isoelectric focussing,
- a gel or polymer to carry out sodium dodecyl sulphate polyacrylamide gel electrophoresis,
- a gel or polymer to perform native gel electrophoresis.
- 20 4. Device for separation (A) according to claim 2 or any one of the above claims, wherein the elution device comprises a frame (11) which borders the eluent (2).
- 25 5. Device for separation (A) according to the preceding claim or any one of the above claims, wherein the frame (11) has compartments (11') for separation of the frame (11) into different compartments, whereby a total volume of eluent (2) contained in the frame (11) is subdivided in portions.
6. Device for separation (A) according to claim 1 or any one of the above

claims, comprising at least one of the features:

the eluent (2) is an appropriate solvent for protein elution;

the elution device is located directly onto the separation medium (1).

7. Device for separation (A) according to claim 1 or any one of the above
5 claims, comprising

a first electrode couple (5) adapted to subject the charged molecules (7) to motion within the separation medium (1), and

at least one second electrode couple (5') adapted to subject the charged molecules (7) to motion from the separation medium (1) into the fluid.

- 10 8. Device for separation (A) according to claim 7, wherein a first electrode (13) of the second electrode couple (5') is immersed in a compartment (11') and a second electrode (14) is in contact with a portion of the separation medium (1) which is located immediately below said compartment (11'), thus being arranged to form an electric circuit when
15 voltage is applied.

9. Device for separation (A) according to claim 1 or any one of the above claims, wherein a plurality of electrode couples (5') is connected in series in order to provide a plurality of compartments (11) with electric circuits in parallel.

- 20 10. Device for separation (A) according to claim 1 or any one of the above claims, wherein said first electrode (13) is covered with a protective film, providing oxidation of charged molecules (7).

11. A processing system (B) for separation and processing of charged molecules (7), comprising at least one device for separation (A) of
25 charged molecules (7) according to claim 1, wherein at least one withdrawing device (20) is provided for withdrawal of a fraction of eluted

molecules.

12. Processing system (B) according to claim 1 or any one of the above claims, wherein a processing device is comprised.
- 5 13. Processing system (B) according to claim 11 or any one of the above claims, wherein the withdrawing device (20) is arranged to immerse in the eluent.
- 10 14. Processing system (B) according to claim 11 or any one of the above claims, wherein the withdrawing device (20) comprises a pipette or a suction hose for transferring the fraction of eluted molecules (7) into the processing device.
- 15 15. Processing system (B) according to claim 12 or any one of the above claims, wherein the processing device comprises a desalting device.
16. Processing system (B) according to claim 12 claim or any one of the above claims, wherein the processing device comprises a digestion device for protein digestion, in particular for trypsin based protein digestion.
17. Processing system (B) according to claim 12 or any one of the above claims, wherein the processing device comprises a device providing concentration of the fraction of eluted molecules (7).
- 20 18. Processing system (B) according to claims 15 to 17the preceding claim or any one of the above claims, wherein the processing device comprises an analytical device, in particular a HPLC-MS device, for characterisation of the eluted molecules.
- 25 19. Processing system (B) according to claim 12 or any one of the above claims, wherein the processing device comprises a separation device for further separation.

20. Processing system (B) according to the preceding claim or any one of the above claims, wherein the device for further separation is an electrophoretic separation device, in particular an electrophoretic separation device based on off-gel electrophoresis.
- 5 21. A method for separation of charged molecules (7) using the device for separation (A) according to claim 1 or any one of the above claims, comprising the steps of:
- applying the molecules (7) to a separation medium (1)
 - setting the molecules (7) into motion within the separation medium (1)
 - 10 to perform electrophoretic separation,
 - bringing the separated molecules (7) in contact with the fluid,
 - setting the charged molecules (7) into motion from the separation medium (1) to the fluid by operating the at least one second electrode (5').
- 15 22. Method according to the preceding claim or any one of the above claims, comprising setting the charged molecules (7) into motion from the separation medium (1) to the fluid providing elution into the eluent (2).
23. Method according to the preceding claim or any one of the above claims, comprising
- 20 - applying voltage on the electrode (5) - "first electrophoresis" -
 - stopping applying voltage on the electrode (5) after a definite operating time
 - performing elution when applying voltage is stopped, thus performing the second step.
- 25 24. Method according to the preceding claim or any one of the above claims, as far as relating to claim 7, wherein said elution is electronically driven by operating at least one of said second electrodes couples (5'), comprising:
- immersing the first electrode (13) of the electrode couple (5') into the

eluent (2),

- placing the second electrode (14) of the electrode couple (5') oppositely to the first electrode (13), thus at the underside of the first separation medium,

5 - applying voltage on said first and second electrodes (13,14) whereby the charged molecules (7) are set into motion from the separation medium (1) into the eluent (2).

25. A method for separating and processing of charged molecules (7) using the processing system (B) according to claim 11 or any one of the above claims, comprising the steps of:

10 - applying the molecules (7) to a separation medium (1)
 - setting the molecules (7) into motion within the separation medium (1) to perform separation,
 - bringing the separated molecules (7) in contact with the fluid,
15 - eluting the charged molecules (7) from the separation medium (1) into the eluent, and
 - withdrawing a fraction of eluted molecules.

26. Method according to the preceding claim or any one of the above claims, comprising transferring the fraction of eluted molecules into a processing device.

27. Method according to claim 25 or any one of the above claims, comprising subjecting of the transferred fraction of eluted molecules to processing steps, in particular to desalting, digesting, concentrating or characterizing.

25 28. Method according to claim 25 or any one of the above claims, comprising subjecting the fraction of eluted molecules to further separation, in particular to electrophoretic separation.

29. Method according to claim 25 or any one of the above claims, wherein

said processing steps are performed automatically.

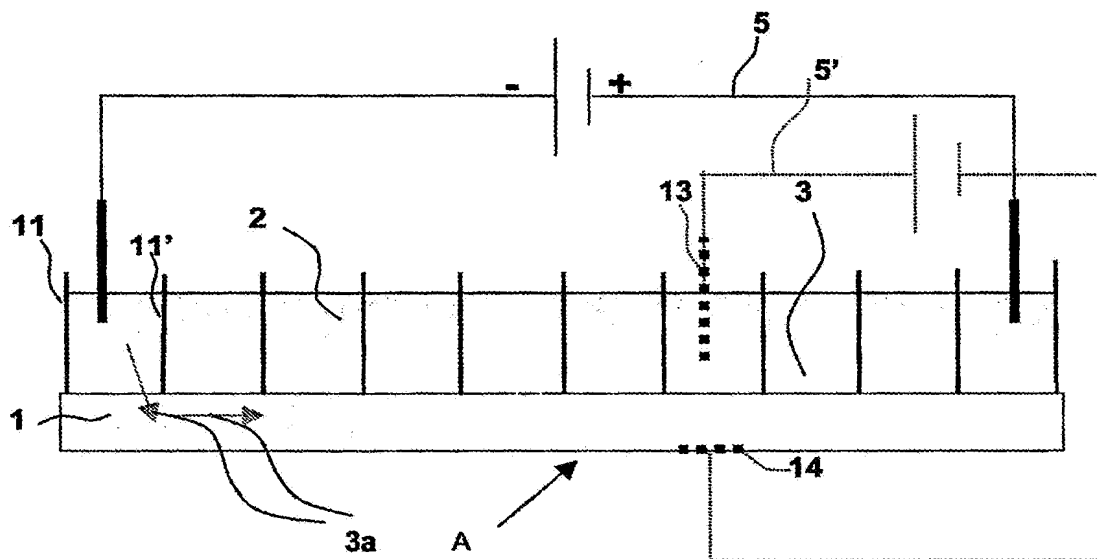


FIG. 1a

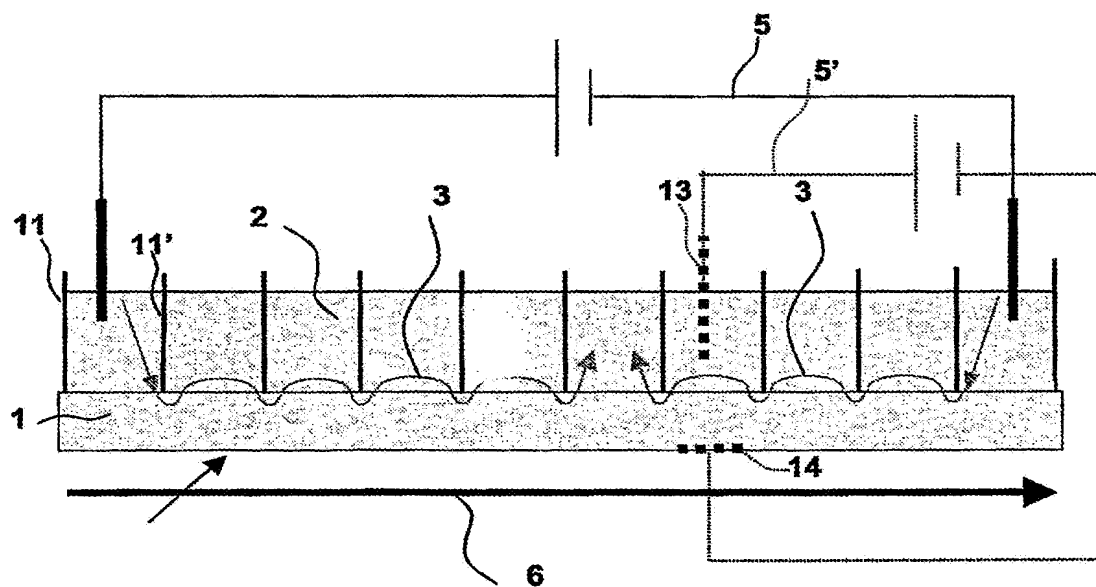


FIG. 1b

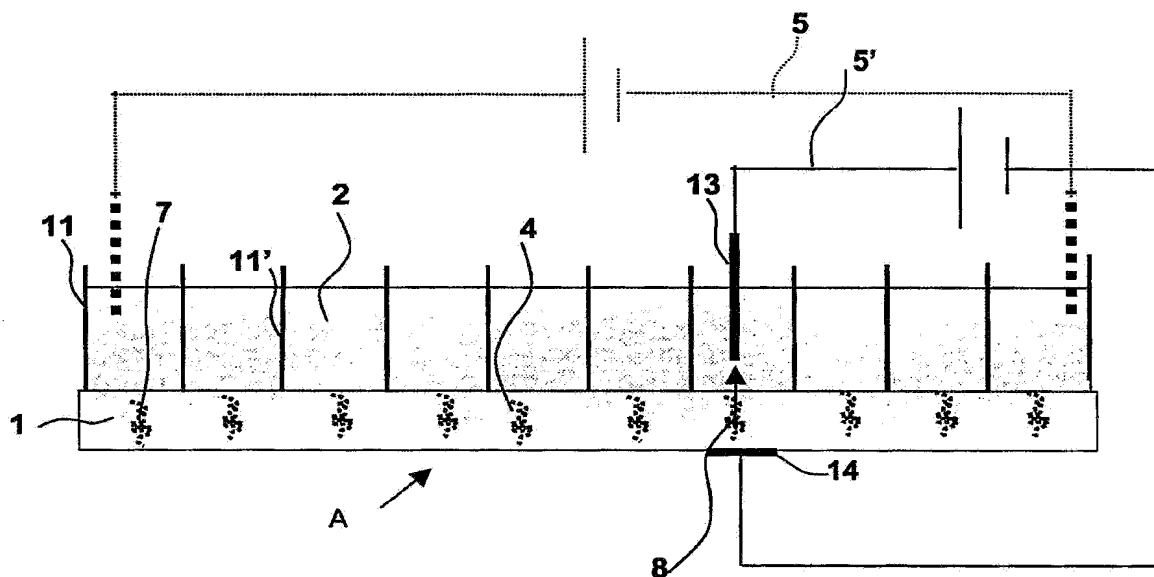


FIG. 2

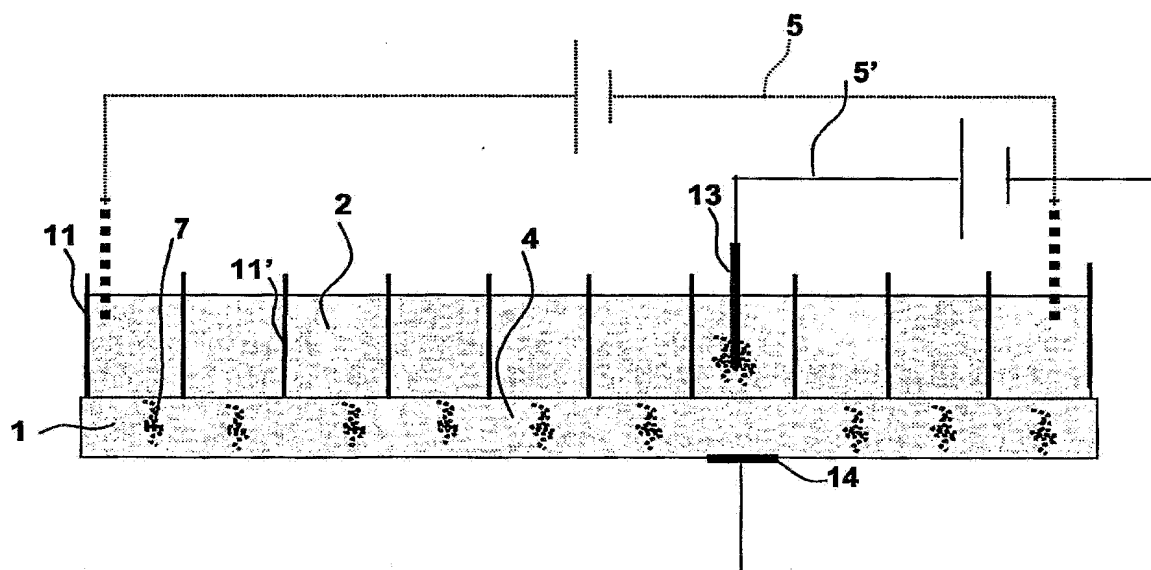


FIG. 3

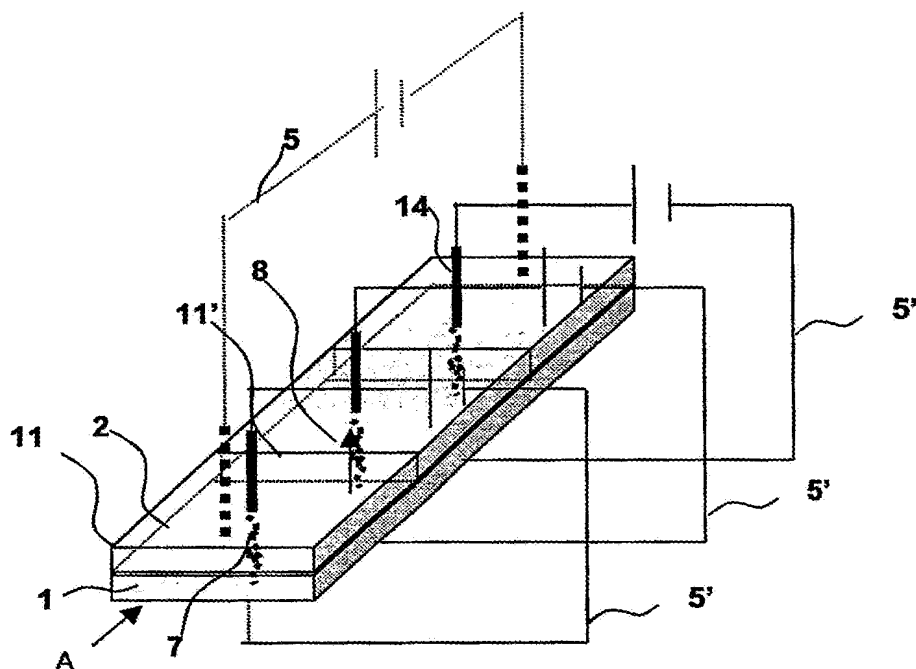


FIG. 4

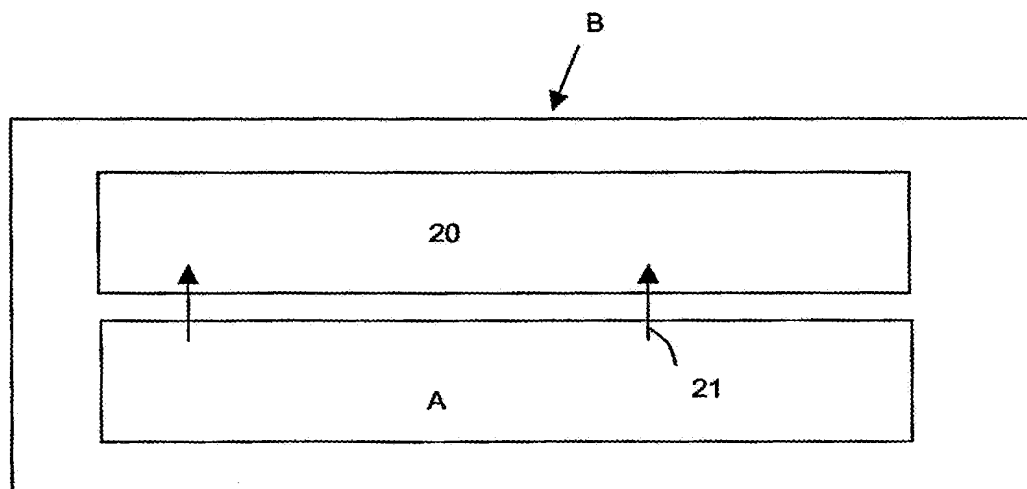


FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/053591

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 B03C5/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B03C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/104449 A1 (FAUPEL MICHEL D ET AL) 5 June 2003 (2003-06-05) cited in the application figures 9,11	1-29
A	WO 03/019172 A (DIAGNOSWISS S.A; MICHEL, PHILIPPE; REYMOND, FREDERIC; ROSSIER, JOEL, S) 6 March 2003 (2003-03-06) cited in the application claim 1	1-29
A	US 5 454 472 A (BENECKE ET AL) 3 October 1995 (1995-10-03) claim 1	1
A	US 2002/036142 A1 (GASCOYNE PETER ET AL) 28 March 2002 (2002-03-28) claim 1	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 August 2005

Date of mailing of the international search report

19/08/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Demol, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/053591

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 2003104449	A1	05-06-2003	AU 6745501 A	20-11-2001
			WO 0186279 A1	15-11-2001
			EP 1281073 A1	05-02-2003
			JP 2003532894 T	05-11-2003
WO 03019172	A	06-03-2003	WO 03019172 A2	06-03-2003
			EP 1421373 A2	26-05-2004
			JP 2005502864 T	27-01-2005
			US 2004231986 A1	25-11-2004
US 5454472	A	03-10-1995	DE 4127405 A1	25-02-1993
			DE 4143573 C2	04-07-1996
			WO 9303850 A1	04-03-1993
			DE 59207522 D1	19-12-1996
			DE 59209969 D1	14-11-2002
			EP 0599957 A1	08-06-1994
			EP 0718038 A2	26-06-1996
			JP 6509745 T	02-11-1994
			JP 3453136 B2	06-10-2003
US 2002036142	A1	28-03-2002	AU 6989601 A	24-12-2001
			CA 2413194 A1	20-12-2001
			EP 1328803 A2	23-07-2003
			JP 2004532968 T	28-10-2004
			WO 0196025 A2	20-12-2001